PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	de Groot, et al.	Examiner:	Graser, J.
Serial No.:	10/049,473	Group Art Unit:	1645
Filed:	July 30, 2002	Docket:	294-120 PCT/US
For:	PNEUMOCOCCAL VACCINES	Dated:	January 7, 2005

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I hereby certify this correspondence is being deposited with the United States Postal Service as first class mail, postpaid in an envelope, addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313 on January 7, 2005
Signature: ____________________

DECLARATION UNDER 37 C.F.R §1.132

Sir;

I, Peter Wilhelmus Maria Hermans, declare as follows:

1. I am one of the inventors for the above-referenced patent application.

2. I am associate professor in Molecular Microbiology within the department of Pediatrics of Erasmus Medical Center Rotterdam - Sophia Children's Hospital, The Netherlands. My curriculum vitae is attached as exhibit 1.

3. I am senior scientist (PhD) and head of the laboratory of Pediatrics within the department of Pediatrics of Erasmus Medical Center Rotterdam - Sophia Children's Hospital, The Netherlands.

4. Currently, I am senior staff member of the department of Pediatrics of Erasmus Medical Center Rotterdam - Sophia Children's Hospital, The Netherlands.

5. This invention relates to an isolated protease maturation protein of *S. Pneumoniae*. The protein contains an amino acid sequence as set forth in SEQ. ID NO: 2, and/or a homologous protein thereof.

6. The term homologous is clearly defined in the specification. Proteins with an E-value (Expect value) of more than 10^{-10}, as determined by Blast or Blastp computer programs, are not considered to be homologous. See the paragraph bridging pages 4 and 5.

7. According to the National Center for Biotechnology Information (NCBI), accessible thru the internet at the url http://www.ncbi.nlm.nih.gov, the Expect value (E) is defined as:

> … a parameter that describes the number of hits one can 'expect' to see just by chance when searching a database of a particular size. It decreases exponentially with the Score (S) that is assigned to a match between two sequences. Essentially, the E value describes the random background noise that exists for matches between sequences. For example, an E value of 1 assigned to a hit can be interpreted as meaning that in a database of the current size one might expect to see 1 match with a similar score simply by chance. This means that the lower the E-value, or the closer it is to "0" the more "significant" the match is. …

8. A copy of the NCBI Blast Frequently Asked Questions (FAQ) which includes the definition of an Expect value is attached as exhibit 2.

9. Kunsch et al. (WO 98/18930) was cited by the examiner in the Office Action. The examiner alleges that Table 1 of Kunsch et al. discloses a polypeptide having 213 identical amino acids to the claimed SEQ. ID NO: 2. Claimed SEQ. ID NO: 2 is 322 amino acids in length. Thus, the examiner concludes that the polypeptide

of Kunsch et al. with this large number of identical amino acids would inherently be homologous to SEQ. ID NO: 2.

10. Black et al. (U.S. Patent No. 6,348,328 B1) was also cited by the examiner. The examiner alleges that Black et al. teaches a polypeptide which has 48 identical amino acids to the claimed SEQ. ID. NO: 2. The examiner asserts that the fragment of Black et al. containing 48 identical amino acids is a homologous sequence.

11. In the previous Office Action (dated October 29, 2003), a sequence comparison between the Kunsch et al. polypeptide and SEQ. ID NO: 2, and a sequence comparison between the Black et al. polypeptide and SEQ. ID. NO: 2 were included. Exhibit 3 is a copy of the Kunsch et al. sequence comparison. Exhibit 4 contains a copy of the Black et al sequence comparison included in the October 23, 2003 Office Action.

12. The sequence comparison demonstrated a 57.7% match between the amino acid sequence of the Kunsch et al. polypeptide and the amino acid sequence of SEQ. ID NO: 2. See exhibit 3. The sequence comparison showed a 20.3% match between the amino acid sequence of the Black et al. polypeptide and the amino acid sequence of SEQ. ID NO: 2. See exhibit 4.

13. It is well known to those skilled in the art that the computer program used for the sequence comparison is not able to calculate an Expect value for comparisons with non-equal sequence lengths. Therefore, Expect values can only be obtained for sequences with equal lengths.

14. Accordingly, for a polypeptide to be considered homologous to SEQ. ID. NO: 2 in accordance with the specification, the polypeptide must also be the same length as SEQ. ID. NO: 2 since Expect values can only be obtained for sequences with equal lengths.

15. Further, it would be apparent to one skilled in the art that, even if the proteins being compared were of equal lengths, such a low percentage match (e.g., 57.7% match for the Kunsch et al. polypeptide and 20.3% match for the Black et al. peptide) would not yield an Expect value that is equal to or less than 10^{-10}, as is required in the claimed invention. Therefore, the polypeptides of Kunsch et al. and Black et al. can not be considered to be homologous to SEQ. ID NO: 2, as is required in the claimed invention.

I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Further that these statements were made with the knowledge that willfully false statements and the like so made are punishable be fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willfully false statements may jeopardize the validity of the application of any patent issued thereon.

Respectfully submitted,

Dated: January 7, 2005

Peter Wilhelmus Maria Hermans

CURRICULUM VITAE

Name	Peter Wilhelmus Maria Hermans
Date of birth	May 6, 1964
Place of birth	Born, The Netherlands
Partner	Hendrika Berends
Children	Sophie Jodith Hermans Anne Marie Hermans
Home address	Bachlaan 204 3261 WC Oud-Beijerland The Netherlands T: 31-186-624038
Office address	Laboratory of Pediatrics / room Ee1500 Erasmus University Rotterdam PO Box 1738 3000 DR Rotterdam The Netherlands T: 31-10-4088224 F: 31-10-4089486 E: hermans@kgk.fgg.eur.nl

Education

High School (VWO-B)
Bisschoppelijk College Sittard
The Netherlands
1976-1982

Biological Sciences (Biochemistry, Microbiology)
University of Nijmegen
The Netherlands
1982-1988

Professional training and academic appointments

1988-1992	Research fellow (PhD student) Unit Molecular Microbiology (Dr. Jan D. A. van Embden), National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands Research subject: Molecular epidemiology and diagnosis of tuberculosis PhD thesis (February 1992): Repeated DNA sequences of *Mycobacterium tuberculosis*: molecular characterization and application in diagnosis and epidemiology of tuberculosis Prof. Dr. W. P. M. Hoekstra (promotor) University of Utrecht The Netherlands
1992-1993	Senior scientist of the Armauer Hansen Research Institute (establishment of a tuberculosis research unit) and associate professor of the Addis Ababa University, Addis Ababa, Ethiopia. Research subject: Molecular epidemiology and immunology of tuberculosis
1993-present	University lecturer, senior scientist and head of the laboratory of Pediatrics (establishment of a molecular biology research unit), department of Pediatrics, Sophia Children's Hospital, Erasmus University Rotterdam, The Netherlands Research subject: Bacterial infectious diseases: molecular epidemiology, molecular pathogenesis and host genetics
2001-present	Director Research and Development Bacterial Vaccines, Vaxinostics Ltd., Erasmus University Rotterdam, The Netherlands

Teaching experience

1988-present	Coaching of many students (over 20) in 6-12 month research projects
1990-present	Lecturer (one week lectures and practicals) of the yearly International Course on Biotechnology, Immunology and Vaccinology (World Health Organization), Geneva/Lausanne, Switzerland
1993-present	Lectures in Microbiology at various universities in The Netherlands
1993-present	Coaching of PhD students (see below)
1995	Lecturer (one week lectures and practicals) in the International Course on the Molecular Epidemiology of Bacterial Infections (BIOLAC program, United Nations University) Caracas, Venezuela
1997	Lecturer (one week lectures) in the International Course on the Molecular Epidemiology of Bacterial Infections (BIOLAC program, United Nations University) La Habana, Cuba
1998	Lecturer of the Boerhaave Course on Pneumococcal Infections and Pneumococcal Vaccination. Leiden, The Netherlands
1999	Lecturer (one week lectures and practicals) of the International Refresher Course on Biotechnology, Immunology and Vaccinology (World Health Organization), Pune, India
1999	Organizer/lecturer (one week lectures and practicals) of the Biotechnology Course on Bacterial Infectious Diseases (sponsored by the Dutch government), Hanoy, Vietnam
2000	Organizer/lecturer (one week lectures and practicals) of the Biotechnology Course on Bacterial Infectious Diseases (sponsored by the Hong Kong government), Hong Kong, China
2001	Lecturer of the Boerhaave Course on Infectious Diseases. Noordwijk, The Netherlands
2002	Organizer/lecturer (one week lectures and practicals) of the Biotechnology Course on Bacterial Infectious Diseases (sponsored by the Dutch government), Ho Chi Min City, Vietnam
2004	Lecturer of the Boerhaave Course on Infectious Diseases. Noordwijk, The Netherlands

Management experience

1993-1998	Acting head of the laboratory of Pediatrics, department of Pediatrics, Sophia Children's Hospital, Erasmus University Rotterdam, The Netherlands
1993-present	Staff member of the department of Pediatrics, Sophia Children's Hospital, Erasmus University Rotterdam, The Netherlands (Chairman of Pediatrics: Prof.dr. H.A. Büller)
1993-present	Board member of the scientific council of the department of Pediatrics, Sophia Children's Hospital, Erasmus University Rotterdam, The Netherlands
1998-present	Head of the laboratory of Pediatrics, department of Pediatrics, Sophia Children's Hospital, Erasmus University Rotterdam, The Netherlands
2000-2001	Secretary of the Dutch Scientific Section on Microbial Pathogenesis
2001-2004	Chairman of the Dutch Scientific Section on Microbial Pathogenesis
2002-2004	Chairman of the scientific board of the Dutch Society for Medical Microbiology

Other activities

1993-present	Reviewer for many international journals, e.g. all ASM Journals, the Journal of Infectious Diseases and Lancet.
1996	Member of the PhD committee of Dr. E.M. Bik (Thesis: Vaccine development and evolution of epidemic *Vibrio cholerae* strains), University of Utrecht, The Netherlands
1996	Member of the PhD committee of Dr. B. Wieles (Thesis: Thioredoxin and thioredoxin reductase of pathogenic mycobacteria), University of Leiden, The Netherlands

1997	Organizer of the Fourth European Meeting on the Molecular Biology of the Pneumococcus, Lunteren, The Netherlands
1997	Member of the European Commission COST/STD Initiative Expert Panel IX: Vaccines against Tuberculosis
1999-2000	Organizer of the scientific program of the Dutch Scientific Section on Microbial Pathogenesis
2001-2002	Member of the scientific advisory board on universal vaccination against meningococcal serogroup C and pneumococcal disease of the Health Council of the Netherlands
2001-present	Member of the editorial board of Lancet Infectious Diseases
2001	Member of the Jury of the Interbrew-Baillet Health Prize 2001 (Fonds National de la Recherche Scientifique, Belgium)
2001	Member of the PhD committee of Dr. H.J. Wisselink (Thesis: *Streptococcus suis* infections in pigs)
2002	Member of the PhD committee of Dr. W.B. van Leeuwen (Thesis: Binary typing of *Staphylococcus aureus*)
2002	Member of the PhD committee of Dr. K. Lagrou (Thesis: Interaction between *Streptococcus pneumoniae* and in vitro human nasopharyngeal epithelium)
2002	Member of the PhD committee of Dr. I.H.M. van Loo (Thesis: Vaccine-driven evolution of *Bortedella pertussis* hanges in population structure and strain fitness)
2003	Member of the PhD committee of Dr. A.W. Rijneveld (Thesis: Pneumonia: an investigation of host defense mechanisms)
2003	Member of the PhD committee of Dr. S. van Selm (Thesis: Generation of capsule diversity in *Streptococcus pneumoniae*)
2003	Member of the PhD committee of Dr. N. de Vries (Thesis: Gene regulation and host adaptation in *Helicobacter pylori*)
2004	Member of the PhD committee of Dr. P.J.G. Zwijnenburg (Thesis: Local inflammatory response in bacterial meningitis). Vrije Universiteit Amsterdam. Promotores: Prof.dr. J.J. Roord, Prof.dr. T. van der Poll. Co-promotor: Dr. A.M. van Furth.
2004	Member of the PhD committee of Dr. R.H. Veenhoven (Thesis: Pneumococcal vaccines for acute otitis media). Universiteit Utrecht. Promotores: Prof.dr..A..M. Sanders, Prof.dr. W. Kuis. Co-promotores: Dr. A.G.M. Schilder, Dr.Ir. G.T. Rijkers.
2004	Member of the PhD committee of Dr. J.L. Nouwen (Thesis: Determinants risks & dynamics of *Staphylococcus aureus* nasal carriage). Erasmus Universiteit Rotterdam. Promotores: Prof.dr. H.A. Verbrugh, Prof.dr. A. Hofman, Prof.dr.dr. A. van Belkum.

PhD theses

Copromotor of Dr. K. Overweg; PhD thesis: *Streptococcus pneumoniae*, molecular epidemiological aspects and the identification of virulence factors (2000; Erasmus University Rotterdam, The Netherlands)
Copromotor of D. Bogaert; PhD thesis: Host-pathogen interaction during *Streptococcus pneumoniae* colonization and infection (2004; Erasmus University Rotterdam, The Netherlands)

Memberships

Dutch Scientific Section on Microbial Pathogenesis
Dutch Society for Microbiology
Dutch Society for Medical Microbiology
Dutch Society for Infectious Diseases
American Society for Microbiology

Grants obtained since 1994

Project	Characterization of the role of adhesion in the pathogenesis of infections caused by *Streptococcus pneumoniae*

Sponsor:	Sophia Foundation for Medical Research (grant no. 177)
Period	1994-1995
Budget	DFL 200,000.-
Project	Molecular epidemiology of infections by *Streptococcus pneumoniae* in children and adults
Sponsor	Sophia Foundation for Medical Research (grant no. 194)
Period	1995-1996
Budget	DFL 200,000.-
Project	Functional and genetic characterization of adhesins involved in the pathogenesis of infections caused by *Streptococcus pneumoniae*
Sponsor	Sophia Foundation for Medical Research (grant no. 183)
Period	1995-1998
Budget	DFL 300,000.-
Project	Non-invasive markers for the diagnosis of Asthma in children
Sponsor	Dutch Asthma Foundation (grant no. 94.14)
Period	1995-1998
Budget	DFL 500,000.-
Project	Genetic characterization of drug-resistant *Streptococcus pneumoniae* in The Netherlands
Sponsor	Sophia Foundation for Medical Research (grant no. 217)
Period	1997-1998
Budget	DFL 200,000.-
Project	Clinical and molecular epidemiological studies of colonization and infection by *Streptococcus pneumoniae* in children and adults, and molecular pathogenesis of pneumococcal infection studied in animal models
Sponsor	Sophia Foundation for Medical Research (grant no. 268) Dutch Science Foundation NWO-SGO (grant no. 005)
Period	1999-2003
Budget	DFL 500,000.- (SSWO) DFL 100,000.- (NWO-SGO)
Project	The protease maturation protein Pmp of *Streptococcus pneumoniae*: immune-protective potentials and its role in the pathogenesis of infection
Sponsor	Sophia Foundation for Medical Research (grant no. 297)
Period	2000-2001
Budget	DFL 250,000.-
Project	Identification of transcriptionally regulated virulence factors and regulatory genes using a *Streptococcus pneumoniae* genome microarray
Sponsor	Sophia Foundation for Medical Research (grant no. 319)
Period	2001-2002
Budget	DFL 200,000.-
Project	Immune protective potential of the putative proteinase maturation protein (PpmA) of *Streptococcus pneumoniae*, fase 1
Sponsor	Dutch Science Foundation NWO-STIGON (Grant no. 014-80-114)
Period	2001-2002
Budget	DFL 300,000.-
Project	Identification of signal networks regulating the expression of virulence genes in *Streptococcus pneumoniae*
Sponsor	Sophia Foundation for Medical Research (grant no. 356)
Period	2002-2005
Budget	€ 250,000.-

Project	Genetic polymorphism within host and pathogen and its relation with infectious diseases and autoimmunity
Sponsor	The Board of the University Hospital Rotterdam (Revolving Fund)
Period	2002-2004
Budget	€ 372,500.-
Project	Development of eradication methods for *Moraxella catarrhalis,* a major cause of otitis media in children
Sponsor	Sophia Foundation for Medical Research (grant no. 397)
Period	2003-2004
Budget	€ 116,860.-
Project	Immune protective potential of the putative proteinase maturation protein (PpmA) of *Streptococcus pneumoniae*, fase 2
Sponsor	Dutch Science Foundation ZON MW-STIGON (grant no. 014-81-114)
Period	2003-2004
Budget	€ 222,000,-
Project	Development of specific monoclonal antibodies for the prevention of nosocomial infections caused by coagulase-negative staphylococci (CNS) in neonates
Sponsor	Erasmus MC, dept. of Pediatrics
Period	2003-2006
Budget	€ 250,000,-
Project	Development and application of genomic array footprinting in *Streptococcus pneumoniae,* a high-throughput technology for genome-wide identification and analysis of essential genes in bacteria
Sponsor	IOP Genomics (Senter)
Period	2004-2007
Budget	€ 650,000,-
Project	Genomics of host-respiratory virus interactions. Towards novel intervention strategies (VIRGO project)
Sponsor	BISK (Senter)
Period	2005-2008
Budget	€ 440.000,- (postdoc for 4 years + laboratory equipment € 120.000,-)
Project	Genetic polymorphisms in host and pathogen associated with infection: chronic hepatitis C (DITTO project)
Sponsor	European Commission, via Prof.dr. S.W. Schalm
Period	2004-2005
Budget	€ 50.000,-

Patents

The international patent application PCT/NL00/00569 entitled 'Pneumococcal Vaccines' has been filed August 14, 2000. A novel patent application, titled 'Method for selecting and producing vaccine components and vaccines based thereon' has been filed May 16, 2003. These patent applications comprise techniques used in the identification of surface-associated pneumococcal proteins, as well as pneumococcal surface proteins and their therapeutic potential.

Publications in international peer-reviewed journals

Van Belkum, A., D. Bogaert, P.W.M. Hermans. Nasopharyngeal colonization with *Staphylococcus aureus* and *Streptococcus pneumoniae* in children is genotype independent. Submitted.

Tonnaer E.L., G.T. Rijkers, J.F. Meis, C.H. Klaassen, D. Bogaert, P.W.M. Hermans, and J.H. Curfs. Genetic relationship between pneumococcal populations originating from nasopharynx, adenoid and tympanic cavity in children with otitis media. Submitted to JID

Menges, T., P.W.M. Hermans, S. G. Little, T. Langefeld, M. Kostrzewa, J. Engel, J. Mühling, K. Weismüller, J. Stricker, E. Nieuwenhuis, M. Emonts, R. de Groot, and G. Hempelmann. Genetic polymorphisms as early predictors for the outcome of severely injured trauma patients. Submitted to Am. J. Respir. Crit. Care Med.

Hays J.P., R. Gorkink, T. Hoogenboezem, G. Simons, K. Eadie, P.W.M. Hermans, C.M. Verduin, H. Verbrugh, and. A. van Belkum. Complement resistant and complement sensitive isolates within the *Moraxella catarrhalis* species actually represent two distinct subspecies. Submitted to App. Env. Microbiol.

Bogaert, D., P.W.M. Hermans, H. Boelens, M. Sluijter, A. Luijendijk, H.C. Rümke, S. Koppen, A. van Belkum, R. de Groot, and H.A. Verbrugh. Epidemiology of nasopharyngeal carriage of *Neisseria meningitidis* in healthy Dutch children. Clin. Infect. Dis, accepted for publication.

Bogaert, D., R.H. Veenhoven, R. Ramdin, I.H.T. Luijendijk, G.T. Rijkers, E.A.M. Sanders, R. de Groot, and P.W.M. Hermans. Pneumococcal conjugate vaccination does not induce persisting mucosal IgA response in children with recurrent acute otitis media. Vaccine, accepted for publication.

Bogaert, D., R.H. Veenhoven, M. Sluijter, W.J.W. Wannet, G.T. Rijkers, T.J. Mitchell, S.C. Clarke, W.H.F. Goessens, A.G. Schilder, E.A.M. Sanders, R. de Groot, and P.W.M. Hermans. 2005. Molecular epidemiology of pneumococcal colonization in response to pneumococcal conjugate vaccination in children with recurrent acute otitis media. J. Clin. Microbiol. *43:*74-83.

Veenhoven, R.H., D. Bogaert, A.G.M. Schilder, G.T. Rijkers, C.S.P.M. Uiterwaal, H.H. Kiezebrink, M.J.P. van Kempen, I.J. Dhooge, J. Bruin, E.P.F. IJzerman, R. de Groot, W. Kuis, P.W.M. Hermans, conjugate and polysaccharide vaccination in children with a history of recurrent acute otitis media. Clin. Infect. Dis. *39:*911-919.

Bogaert, D., M. Sluijter, R. de Groot, and P.W.M. Hermans. 2004. Multiplex opsonophagocytosis assay (MOPA): a useful tool for the monitoring of the 7-valent pneumococcal conjugate vaccine. Vaccine *22:*4014-4020.

Adrian, P.V., D. Bogaert, M. Oprins, S. Rapola, M. Lahdenkari, T. Kilpi, R. de Groot, H. Käyhty, and P.W.M. Hermans. 2004. Development of antibodies against pneumococcal proteins α-Enolase, immunoglobulin A1 protease, streptococcal lipoprotein rotamase A, and putative proteinase maturation protein A in relation to pneumococcal carriage and Otitis Media. Vaccine *22:*2737-2742.

Kerr, A.R., P.V. Adrian, S.C. Estevão, R. de Groot, G. Alloing, J.P. Claverys, T.J. Mitchell, and P.W.M. Hermans. 2004. The Ami-AliA/AliB permease of *Streptococcus pneumoniae* is involved in nasopharyngeal colonization but not in invasive disease. Infect. Immun. *72:*3902-3906.

Bogaert, D., R.H. Veenhoven, M. Sluijter, E.A.M. Sanders, R. de Groot, and P.W.M. Hermans. 2004. Colony blot assay: a useful method to detect multiple pneumococcal serotypes within clinical specimens. FEMS Immunol. Med. Microbiol. *41:*259-264.

Bogaert, D., A. van Belkum, M. Sluijter, A. Luijendijk, R. de Groot, H.C. Rümke, H.A. Verbrugh, and P.W.M. Hermans. 2004. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. Lancet *363:*1871-1872.

Bogaert, D., P.W.M. Hermans, P.V. Adrian, H.C. Rümke, and R. de Groot. 2004. Pneumococcal vaccines: an update on current strategies. Vaccine *22:*2209-2220.

Emonts, M., R. de Groot, and P.W.M. Hermans. 2004. Genetic susceptibility to *Neisseria meningitidis* infections. Neth. J. of Med. *62:*(S)28-37.

Bogaert, D., P. van der Valk, R. Ramdin, M. Sluijter, E. Monninkhof, R. Hendrix, R. de Groot, and P.W.M. Hermans. 2004. Host-pathogen interaction during pneumococcal infection in patients with chronic obstructive pulmonary disease. Infect. Immun. *72*:818-823.

Haralambous, E., M.L. Hibberd, P.W.M. Hermans, N. Ninis, S. Nadel, and M. Levin. 2003. Role of the functional plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism in susceptibility, severity, and outcome of meningococcal disease in Caucasian children. Crit. Care Med. *31*:2788-2793.

Bogaert, D., P.W.M. Hermans, I.N. Grivea, G.S. Katopodis, T.J. Mitchell, M. Sluijter, R. de Groot, N.G. Beratis, and G.A. Syrogiannopoulos. 2003. Molecular epidemiology of penicillin-susceptible non-ß-lactam-resistant *Streptococcus pneumoniae* isolates from Greek children. J. Clin. Microbiol.; *41:* 5633-5639.

Sanders, E.A.M., R. Veenhoven, D. Bogaert, A. Schilder, and P.W.M. Hermans. 2003. Effect of conjugate pneumococcal vaccine on recurrent acute otitis media. Lancet; *362:*1081.

Veenhoven, R., D. Bogaert, C. Uiterwaal, C. Brouwer, H. Kiezebrink, J. Bruin, E. IJzerman, P.W.M. Hermans, R. de Groot, B. Zegers, W. Kuis, G. Rijkers, A. Schilder, and E.A.M. Sanders. 2003. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media. Lancet;*361*:2189-2195.

Bogaert, D., N.T. Ha, M. Sluijter, N. Lemmens, R. de Groot, and P.W.M. Hermans. 2002. Molecular epidemiology of pneumococcal carriage among children with upper respiratory tract infections in Hanoi, Vietnam. J. Clin. Microbiol. *40:*3903-3908.

Overweg, K., D. Bogaert, M. Sluijter, R. de Groot, and P.W.M. Hermans. 2001. Molecular characteristics of penicillin-binding protein genes of penicillin-nonsusceptible *Streptococcus pneumoniae* isolated in The Netherlands. Microb. Drug. Resist. *7*:323-334.

Bogaert, D., M.N. Engelen, A.J.M. Timmers-Reker, K.P. Elzenaar, P.G.H. Peerbooms, R.A. Coutinho, R. de Groot, and P.W.M. Hermans. 2001. Pneumococcal carriage in children in The Netherlands: a molecular epidemiological study. J. Clin. Microbiol. *39:*3316-3320.

Van Tilburg, P.M.B., D. Bogaert, M. Sluijter, A.R. Jansz, R. de Groot, and P.W.M. Hermans. 2001. Emergence of rifampicin-resistant *Streptococcus pneumoniae* as a result of antimicrobial therapy for penicillin-resistant strains. Clin. Infect. Dis. *33:*e93-e96.

Menges, T., P.W.M. Hermans, S.G. Little, T. Langefeld, O. Böning, J. Engel, M. Sluijter, R. de Groot, and G. Hempelmann. 2001. Plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism and prognosis of severely injured patients. Lancet *357:*1096-1097.

Syrogiannopoulos, G.A., D. Bogaert, I.N. Grivea, N.G. Beratis, R. de Groot, and P.W.M. Hermans. 2001. Molecular epidemiology of penicillin-susceptible, multidrug-resistant serotype 6B pneumococci isolated from children in Greece. J. Clin. Microbiol. *39:*581-585.

Overweg, K., D. Bogaert, M. Sluijter, J.L. Yother, J. Dankert, R. de Groot, and P.W.M. Hermans. 2000. Genetic relatedness within serotypes of penicillin-susceptible *Streptococcus pneumoniae* isolates. J. Clin. Microbiol. *38:*4548-4553.

Bogaert, D., G.A., Syrogiannopoulos, I.N. Grivea, R. de Groot, N.G. Beratis, and P.W.M. Hermans. 2000. Molecular epidemiology of penicillin-nonsusceptible *Streptococcus pneumoniae* in Greece. J. Clin. Microbiol. *38:*4361-4366.

Goessens, W.H.F., N. Lemmens-den Toom, J. Hageman, P.W.M. Hermans, M. Sluijter, R. de Groot, and H.A. Verbrugh. 2000. Evaluation of the VITEC 2 system for susceptibility testing of *Streptococcus pneumoniae* isolates. Eur. J. Clin. Microbiol. Inf. Dis. *19:*618-622.

Overweg, K., M. Sluijter, M. Srodzinski, R. de Groot, and P.W.M. Hermans. 2000. Immune-protective antibodies against capsular polysaccharides do not affect natural competence of *Streptococcus*

pneumoniae: implications for current conjugate vaccination strategies? FEMS Immunol. Med. Microbiol. *29:*183-185.

Overweg, K., C.D. Pericone, G.G.C. Verhoef, J.N. Weiser, H.D. Meiring, A.P.J.M. de Jong, R. de Groot, and P.W.M. Hermans. 2000. Differential protein expression in phenotypic variants of *Streptococcus pneumoniae*. Infect. Immun. *68:*4604-4610.

Overweg, K., A. Kerr, M. Sluijter, M.H. Jackson, T.J. Mitchell, A.P.J.M. de Jong, R. de Groot, and P.W.M. Hermans. 2000. The putative proteinase maturation protein A of *Streptococcus pneumoniae* is a conserved surface protein with potential to elicit protective immune responses. Infect. Immun. *68:*4180-4188.

Pericone, C.D., K. Overweg, P.W.M. Hermans, and J.N. Weiser. 2000. Inhibitory and bactericidal effects of hydrogen peroxide production by *Streptococcus pneumoniae* on other inhabitants of the upper respiratory tract. Infect. Immun. *68:*3990-3997.

Hermans, P.W.M., T. Hoogenboezem, and R. de Groot. 2000. Adaptation and survival of *Mycobacterium tuberculosis* in the host. J. Clin. Virol. *16:*84-85.

De Galan, B.E., P.M.B. van Tilburg, M. Sluijter, S.J.M. Mol, R. de Groot, P.W.M. Hermans, and A.R. Jansz. 1999. Hospital-related outbreak of infection with multidrug-resistant *Streptococcus pneumoniae* in The Netherlands. J. Hosp. Infect. *42:*185-192.

Dejsirilert, S., K. Overweg, M. Sluijter, L. Saengsuk, M. Gratten, T. Ezaki, and P.W.M. Hermans. 1999. Nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* among children with acute respiratory tract infections in Thailand: a molecular epidemiological survey. J. Clin. Microbiol. *37:*1832-1838.

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BLAST Frequently Asked Questions (FAQ)

Tips and hints

Q: Which BLAST program should I use?

You have many choices to make between different BLAST programs and databases. Some of these choices are better for answering some questions then others. We have created a selection chart to help you make the decision of BLAST program for the question you are asking. This is the "BLAST Program Selection Guide".

Q: How can I search a batch of sequences with BLAST?

There are three options for "Batch" BLAST searches:
1) **Web MegaBLAST EST analysis tool:** This program is optimized for aligning nucleotide sequences that differ slightly as a result of sequencing or other similar "errors". MegaBLAST is good for scanning a large number of EST type sequences (about 500 kb in length) against large database in search of the closest matches. You can import a file EST sequences in FASTA format or as a list of GenBank accessions or/GIs and have them compared to the BLAST databases. The default is an easily reviewable Hit Table format, although you can download and save the results in Standard pairwise HTML or any of the other result output options. MegaBLAST is available from the BLAST web page, the standalone BLAST executables, or

via the network BLAST client (see below).

2) **Standalone BLAST executables:** The Standalone BLAST executables are command line programs which run BLAST searches against local downloaded copies of the NCBI BLAST databases. The programs will handle either a single large file with multiple FASTA query sequences, or you can create a script to send multiple files one at a time. The executables are available for a wide variety of platforms, including many "flavors" of UNIX (LINUS, Solaris, etc.) Windows PC and even Mac OSX.

The Standalone executables are available at the anonymous FTP location: ftp://ftp.ncbi.nih.gov/blast/executables/ There is information on the Standalone BLAST executables available in the README file at ftp://ftp.ncbi.nih.gov/blast/documents/blast.txt which is also bundled with the downloaded binaries.

3) **BLAST Network Client 'blastcl3':** The BLAST 2.0 Network client will allow you to submit a single file of FASTA sequences over an internet connection to the NCBI BLAST databases. You submit searches through the client to the NCBI servers and do not need to download the database locally. The BLAST Network client executables are located at: ftp://ftp.ncbi.nlm.nih.gov/blast/executables/ There are blastcl3 executables for various UNIX platforms, PC Windows and Macintosh.

Q: How can I write a program to submit jobs to NCBI's BLAST servers?

By using the URLAPI. Documentation also available in postscript and PDF.

Q: How can I limit my BLAST search based on Organism?

The option to limit a search to organism and even taxonomic classification is part of the "Limit by Entrez Search" option on most standard BLAST search pages. There is a pull down menu to select the most common organisms found in GenBank and also a field to input the species name, or classification (example: "eubacteria"). Using this option will cause your query sequences to be compared only to sequences in our databases from that organism.

There are also several "specialized" BLAST Pages devoted to different organisms on the main BLAST web page.

How can I limit my search to a subset of database sequences?

You can use the "Limit by Entrez Search" option found on most Standard BLASTR search pages to run an Entrez search and have your query sequence compared to the resutls of this search. For example, if you wanted to limit you search to all phosphorylase sequences from mouse you could enter the following valid Entrez search strategy in the Limit by Entrez field of the BLAST search page: phosphorylase AND "Mus musculus"[Organism]

Q: Is it possible to search for a motif or pattern with BLAST?

There are two general approaches to this type of questions. First do you wish to find if motifs exist in your query sequence, or do you have a known motif and wish to find other protiens or nucleotides with this motif?

In the first case, finding motifs in your query sequence can be done for proteins using the CDD (Conserved Domain Database) and CDART (Conserved Domain Architecture Retrieval Tool) tools. CDD allows you to compare your protein to an database of alignments and profiles

representing protein domains conserved in molecular evolution as well as 3-dimensional protein structures in the MMDB database. These tools use popular protien motif databases, PFam (http://pfam.wustl.edu/) and Smart (http://smart.embl-heidelberg.de) in addition to the MMDB database.

For conditions of the second case if you have a known motif and wish to identify other proteins with this motif you can use PHI-BLAST. PHI-BLAST searches take a motif pattern and protein sequence as input and then compares these to the NCBI protein databases looking for other proteins which contain conserved regions similar to the motif entered.

For nucleotides it is only possible to search with short query sequences representing your motif or region of interest with the Nucleotide BLAST "Search for short nearly exact matches" service from the main BLAST web page. This can find other sequences whicvh contain similar nucleotide patterns. however there are no database of nucleotide patterns which can identify patterns in your nucleotide query sequence.

You may also be interested in checking out other molecular biology web sites, such as those mentioned in the Other Molecular Biology Resources section at the end of this FAQ, for motif searching software.

Q: How do I perform a similarity search with a short peptide/nucleotide sequence?

There is a special page with pre-set parameters for searching with short sequences. You can access this page by clicking the "Search for short nearly exact matches" link on the main BLAST web page.

Essentially for these searches, the Expect value has been increased and the word size decreased to optimise for short hits which generally score a large E value require smaller word sizes to initiate formation of the HSP for extension. In addition, for proteins, the matix "PAM30" becomes the default which optimises hits to smaller sequences which have a lower percentage of evolutionary drift in general.

Q: Can I use BLAST to compare to two or more sequences in a multiple sequence alignment?

You can use the BLAST 2 Sequences service to compare two nucleotide or two protein sequences against each other using the Gapped BLAST algorithm. The this will allow you to perform a BLAST search between the two sequences allowing for the introduction of gaps (deletions and insertions) in the resulting alignment. Remember that BLAST is a "local" alignment program and does not make global alignments between sequences to calculate total percent homologies.

To compare one sequence against a specific sequence or set of sequences, you can also use a separate multiple sequence alignment program. There are many such software tools available to do this. You may also be interested in checking out other molecular biology web sites, such as those mentioned in the Other Molecular Biology Resources section at the end of this FAQ.

Q: What is the Expect (E) value?

The Expect value (E) is a parameter that describes the number of hits one can "expect" to see just by chance when searching a database of a particular size. It decreases exponentially with the Score (S) that is assigned to a match between two sequences. Essentially, the E value

describes the random background noise that exists for matches between sequences. For example, an E value of 1 assigned to a hit can be interpreted as meaning that in a database of the current size one might expect to see 1 match with a similar score simply by chance. This means that the lower the E-value, or the closer it is to "0" the more "significant" the match is. However, keep in mind that searches with short sequences, can be virtually indentical and have relatively high EValue. This is because the calculation of the E-value also takes into account the length of the Query sequence. This is because shorter sequences have a high probability of occurring in the database purely by chance. For more details please see the calculations in the BLAST Course.

The Expect value can also be used as a convenient way to create a significance threshold for reporting results. You can change the Expect value threshold on most main BLAST search pages. When the Expect value is increased from the default value of 10, a larger list with more low-scoring hits can be reported.

Q: What is low-complexity sequence?

Regions with low-complexity sequence have an unusual composition andthis can create problems in sequence similarity searching (Wootton & Federhen, 1996). Low-complexity sequence can often be recognized by visual inspection. For example, the protein sequence PPCDPPPPPKDKKKKDDGPP has low complexity and so does the nucleotide sequence AAATAAAAAAAATAAAAAAT. Filters are used to remove low-complexity sequence because it can cause artifactual hits (please also see Q: After running a search why do I see a string of "X"s (or "N"s) in my query sequence that I did not put there?)

In BLAST searches performed without a filter, often certain hits will be reported with high scores only because of the presence of a low-complexity region. Most often, this type of match cannot be thought of as the result of homology shared by the sequences. Rather, it is as if the low-complexity region is "sticky" and is pulling out many sequences that are not truly related.

Other Molecular Biology Resources:

The on-line **BLAST Course** was written by Dr. Stephen Altschul and discusses the basics of the Gapped BLAST algorithm. In addition the **full text** of the 1997 Nucleic Acids Research paper "**Gapped BLAST and PSI-BLAST: a new generation of protein database search programs**" is also available on-line.

Other links:

- European Bioinformatics Institute (EBI) BioCatalog
- Indiana University IUBio Archive
- Sequence manipulation site

Troubleshooting

Q: Causes for "No significant similarity found".

Below are several reasons that a BLAST search can result in the "No significant similarity found" message.

Short Sequences: There is a special BLAST optimized for searchig with small sequences. Go to tbe main BLAST web page and select the "**Search for short nearly exact matches**" link for Nucleotide - Nucleotide or Protein Protein sections.

Filtering: BLAST filters regions of low-complexity (for a description of low-complexity see "What is low-complexity sequence?" below). If your sequence contains large regions of "low complexity" it may not significant hits to the database. You can turn off filtering by setting the "**Filter**" option to "**None**" using the pull down tab.

Query Format: Another reason you may see the "No Significant Similarity found" message is using the wrong type of sequence in your search.

1) Accession/GI Number or FASTA. Check that you have the Input Data set to the correct format for your Query. Set the pull down menu to "Accession number or Gi" to search with GenBank accession numbers or Gi numbers. Set to FASTA for raw amino acid or nucleotide sequences. For more information on FASTA format, click here.

2) Sequence type and Program combination. You can search with an amino acid query sequence using the blastp and tblastn programs. With nucleotide query sequences you can use blastn, blastx, and tblastx. Please note that tblastx program cannot be used with the nr database on the BLAST Web page.

For more information on the BLAST programs, click here.

Q: Why does my search timeout on the BLAST servers?

Certain combinations of BLAST searches with large sequences against large databases can cause the BLAST servers to timeout. This has to do with a limit on the server CPU's which prevents sequences which generate many HSPs from hoarding server resources.

However there are some things you can do to prevent timeout and generate results from large sequences.

- Some sequences contain large regions of ALU repeats. In this case you can select the "Human Repeat" filtering option on the main BLAST search page. This will mask repeat regions which generate a large number of biologically uninteresting hits to the databases.

- Increase the Word Size to 20 - 25. With a default Word Size of 7, the BLAST algorithm finds initial HSPs of 7 bases in length and begins extension of these from either end. In a large sequence this can generate 100's of initial HSPs between the query sequence and even a single large genomic sequence in the databases. Increasing the Word Size to 25 makes the initial HSP smaller, limiting the number small initial fragments to be extended.

- Decrease the Expect value to 1.0 or lower. Many hits from large sequences are to many small fragments in the database. The expect value for these searches is such that decreasing the expect value will eliminate these results, and concentrate on results which are more likely to contain large coding regions and genomic fragments.

If you are still seeing a "timeout" error message after making the above changes, please contact blast-help@ncbi.nlm.nih.gov with the RID of your search.

Q: Why do I get the message "ERROR:BLASTSetUpSearch: Unable to calculate Karlin-Altschul params, check querysequence" ?

This will happen if your entire query sequence has been masked by low complexity filtering. You will need to turn filtering off to get hits. For further information on filtering, please read the sections of the BLAST FAQs on Q: What is low-complexity sequence? and also Q: After

running a search why do I see a string of "X"s (or "N"s) in my query sequence that I did not put there?

Q: Why do I get the message "ERROR: Blast: No valid letters to be indexed"?

You may have accidentally entered an accession number in the search box without changing the input selection from "Sequence in FASTA format" to "Accession or gi". You will also see this error message if too many ambiguity codes (R,Y,K,W,N, etc. fornucleotides) are present in your query sequence. Although BLAST allows ambiguity codes, be aware that these will always contribute a negative score in nucleic acid searches. Thus, sequences such as degenerate PCR primers with ambiguity codes maynot find any significant hits even though they may be designed from sequences that are present in the database.

Q: After running a search why do I see a string of "X"s (or "N"s) in my query sequence that I did not put there?

You are seeing the result of automatic filtering of your query for low-complexity sequence that is performed to prevent artifactual hits. The filter substitutes any low-complexity sequence that it finds with the letter "N" in nucleotide sequence (e.g., "NNNNNNNNNNNNNNN") or the letter "X" in protein sequences (e.g., "XXXXXXXXXX"). Low-complexity regions can result in high scores that reflect compositional bias rather than significant position-by-position alignment (Wootton amp; Federhen, 1996). Filter programs can eliminate these potentially confounding matches from the blast reports, leaving regions whose BLAST statistics reflect the specificity of their parities alignment. Queries searched with the blastn program are filtered with DUST. The other BLAST programs use SEG.

Q: How can I see low-similarity matches when there are many strong hits to my query sequence? Often, when the query is a member of a large sequence family, the summary hit list and the alignments returned only contain very high scoring hits. To look at low-similarity matches, you must increase the maximum number of results returned. On the BLAST Web pages, often it is sufficient to increase the size of the summary hit list and the number of alignments shown using the menus on the Advanced pages. However, it is possible to increase the lists even further using the Other Advanced Options box on the Advanced BLAST pages. For BLAST 2.0, "-v 2000", for example, will increase the number of descriptions returned in the summary hit list to 2000. The option "-b 2000" will similarly increase the number of alignments returned.

Q: I have heard that I will be penalized if I send a large number of sequences to the servers? .

The NCBI WWW BLAST server is a shared resource and it would be unfair for a few users to monoplize it. To prevent this, the server keeps track of how many queries are in the queue for each user and penalzies those users with many queries in the queue. This is done by calculating a 'Time of Execution' (TOE). If a user has only one query in the queue, then the TOE is set to the current time. As a user adds more queries to the queue, then the TOE is set to the current time, plus 60 seconds for every query in the queue. An example would be if a user sent in five requests one after the other without waiting for any to be worked on, then the TOE's for the requests would be:

1st request: current time

2nd request: current time + 60 seconds

3rd request: current time + 120 seconds

4th request: current time + 180 seconds
5th request: current time + 240 seconds

The BLAST server works through requests in the order of earliest to latest TOE. A query will be executed before it's TOE, if there are no other queries with an earlier TOE. Users with large numbers of queries are encouraged to use the BLAST servers at off-peaks hours, which are from 8 p.m. to 8 a.m. (EST).

```
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
b        241 KKTEKSSNIDDYKEKLKTVILTQKQNDSTFVQSIIGKELQAANIKVKDQAFQNIFTQYIG 300

y        310 GGDSSSSSSTSNE 322
             |||||||||||||
b        301 GGDSSSSSSTSNE 313
```

ESULT 4
AW55079
D   AAW55079 standard; Protein; 213 AA.
X
C   AAW55079;
X
T   OCT-1998 (first entry)
X
E   eptococcus pneumoniae SP0021 protein.
X
W   Streptococcus pneumoniae; antigen; vaccine; infection; diagnosis;
W   detection; pneumonia; otitis media; meningitis.
X
S   Streptococcus pneumoniae.
X
N   WO9818930-A2.
X
D   07-MAY-1998.
X
F   30-OCT-1997; 97WO-US19422.
X
R   31-OCT-1996; 96US-0029960.
X
A   (HUMA-) HUMAN GENOME SCI INC.
X
I   Choi GH, Hromockyj A, Johnson LS, Kunsch CA;
X
R   WPI; 1998-272224/24.
R   N-PSDB; AAV27340.
X
T   Nucleic acid encoding antigenic peptide(s) from Streptococcus
T   pneumoniae - or their epitope-containing fragments, useful in
T   protective or therapeutic vaccines, and for diagnosis
X
S   laim 11; Page 55; 118pp; English.
X
C   present sequence represents a protein from Streptococcus pneumoniae.
C   e nucleic acid sequence encoding the Streptococcus pneumoniae protein
C   can be useful in vaccines for inducing protective antibodies against
C   Streptococcus pneumoniae, for treatment or prevention of infection e.g.
C   pneumonia, otitis media or meningitis. Probes based on the nucleic acid
C   are used to detect Streptococcus infection (by usual hybridisation or
C   amplification methods), also for isolating Streptococcus genes or their
C   allelic variants. The protein can be used similarly to detect specific
C   antibodies in standard immunoassays, especially for diagnosing or
C   monitoring infections. Antibodies which bind the protein are used to
C   detect corresponding antigens, to purify the protein and for passive
C   immunisation (optionally coupled to a toxin). Vaccines are administered,
C   e.g. by injection, orally or through the skin, typically at 0.01-1000
C   (especially 10-300) mu g/ml per dose.
X
Q   Sequence 213 AA;

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```
Query Match             57.7%;  Score 916;  DB 19;  Length 213;
Best Local Similarity   93.6%;  Pred. No. 3.3e-62;
Matches  190;  Conservative    0;  Mismatches    5;  Indels    8;  Gaps    2;

Qy        31 SKGSEGADLISMKGDVITEHQFYEQVKSNPSAQQVLLNMTIQKVFEKQYGSELDDKEVDD 90
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         1 SKGSEGADLISMKGDVITEHQFYEQVKSNPSAQQVLLNMTIQKVFEKQYGSELDDKEVDD 60

Qy        91 TIAEEKKQYGENYQRVLSQAGMTLETRKAQIRTSKLVELAVKKVAEAELTDEAYKKAFDE 150
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        61 TIAEEKKQYGENYQRVLSQAGMTLETRKAQIRTSKLVELAVKKVAEAELTDEAYKKAFDE 120
```

309 GG... ||||... ...TSN 2...

us-10-049-473a-2

Fri Sep 5 09:24:50 2003

us-10-049-

```
Qy       151 YTPDVTAQIIRLNNEDKAKEVLEKAKAEGADFAQLAKDNSTDEKTKENGGEITFDSASTE 210
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       121 YTPDVTAQIIRLNNEDKAKEVLEKAKAEGADFAQLAKDNSTDEKTKENGGEITFDSASTE 180

Qy       211 VP-EQVKKAAFA-------LDVD 225 322
             ||   ||  ||        ||||
Db       181 VPGASPKKPLFAFRCGMVFLDVD 203
```

RESULT 5
ABP54573
ID   ABP54573 standard; Protein; 213 AA.
XX
AC   ABP54573;
XX
DT   04-SEP-2002 (first entry)
XX
DE   S. pneumoniae SP021 protein sequence SEQ ID NO:34.
XX
KW   Streptococcus pneumoniae; epitope; vaccine; antigenic protein;
KW   antibacterial; Streptococcal infection; detection.
XX
OS   Streptococcus pneumoniae.
XX
PN   US2002061545-A1.
XX
PD   23-MAY-2002.
XX
PF   22-JAN-2001; 2001US-0765272.
XX
PR   30-OCT-1997; 97US-0961083.
XX
PA   (CHOI/) CHOI G H.
PA   (KUNS/) KUNSCH C A.
PA   (BARA/) BARASH S C.
PA   (DILL/) DILLON P J.
PA   (DOUG/) DOUGHERTY B.
PA   (FANN/) FANNON M R.
PA   (ROSE/) ROSEN C A.
XX
PI   Choi GH, Kunsch CA, Barash SC, Dillon PJ, Dougherty B, Fannon MR;
PI   Rosen CA;
XX
DR   WPI; 2002-479261/51.
DR   N-PSDB; ABQ84808.
XX
PT   New Streptococcus pneumoniae antigens, useful for detecting
PT   Streptococcus and for preventing or attenuating disease caused by
PT   Streptococcus infection -
XX
PS   Claim 11; Page 24; 70pp; English.
XX
CC   ABQ84792 to ABQ84904 represents nucleic acids which encode the
CC   Streptococcus pneumoniae antigens given in ABP54557 to ABP54669.
CC   The S. pneumoniae antigens have antibacterial activity and can be
CC   used in vaccines. The S. pneumoniae antigens can also be used to
CC   prevent or attenuate a Streptococcal infection in an animal. The
CC   polynucleotides encoding the S. pneumoniae antigens can be used to
CC   detect Streptococcus nucleic acids. ABQ84905 to ABQ85130 represent
CC   primers used in the cloning of S. pneumoniae ORFs (open reading frames)
CC   which are used in an example from the present invention.
XX
SQ   Sequence 213 AA;

```
                        57.7%;  Score 916;  DB 23;  Length 213;
             ilarity    93.6%;  Pred. No. 3.3e-62;
             onservative    0;  Mismatches    5;  Indels    8;  Gaps    2;

             LISMKGDVITEHQFYEQVKSNPSAQQVLLNMTIQKVFEKQYGSELDDKEVDD 90
             ||||||||||||||||||||||||||||||||||||||||||||||||||||
```

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```
Qy        70 TIQKVFEKQYGSELDDKEVDDTIAEEKKQYGENYQRVLSQAGMTLETRKAQIRTSKLVEL 129
Db        70 IVFKAFDDVYGKEISSKEVTKEYNEQIKKLGPNYKEQLKAVGQTEETYKKLFKQMLAFQY 129

Qy       130 AVKKVAEAELTDEAYKKAFDEYTPDVTAQIIRLNNEDKAKEVLEKAKAEGADFAQLAKDN 189
Db       130 GLK--ANVKLTDKDLDTAWKEFYPEVSTQIILFSTKEEADKAKKEAN-EGENFSKLVQAY 186

Qy       190 STDEKTKENGGEITFDSASTEVPEQVKKAAFALDVDGVSDVITATGTQAYSSQYYIVKLT 249
Db       187 GKNKTLKETDGKMNFDSTNPEIPTEVKKAAFKLKNGEVSDIIPVTDPTTYQQSYYLVKMV 246

Qy       250 KKTEKSSNIDDYKEKLKTVILTQKQNDSTFVQSIIGKELQAANIKVKDQAFQNIF 304
Db       247 KKQDKGSNKDKYKSELEKIATEAKTLDTEFMDKTIRKVMKKDNVTIKDPYVKNIF 301
```

ULT 8
08-858-207A-508
; Sequence 508, Application US/08858207A
; Patent No. 6348328
; GENERAL INFORMATION:
; APPLICANT: Black, Michael
; APPLICANT: Hodgson, John
; APPLICANT: Knowles, David
; APPLICANT: Nicholas, Richard
; APPLICANT: Stodola, Robert
; TITLE OF INVENTION: No. 6348328el Compounds
; NUMBER OF SEQUENCES: 552
; CORRESPONDENCE ADDRESS:
; ADDRESSEE: SmithKline Beecham Corporation
; STREET: 709 Swedeland Road
; CITY: King of Prussia
; STATE: PA
; COUNTRY: USA
; ZIP: 19406-0939
; COMPUTER READABLE FORM:
; MEDIUM TYPE: Diskette
; COMPUTER: IBM Compatible
; OPERATING SYSTEM: DOS
SOFTWARE: FastSEQ for Windows Version 2.0
CURRENT APPLICATION DATA:
APPLICATION NUMBER: US/08/858,207A
FILING DATE: 09-MAY-1997
CLASSIFICATION: 435
PRIOR APPLICATION DATA:
APPLICATION NUMBER: 60/017670
FILING DATE: 14-MAY-1996
ATTORNEY/AGENT INFORMATION:
NAME: Gimmi, Edward R
REGISTRATION NUMBER: 38,891
REFERENCE/DOCKET NUMBER: P50475
TELECOMMUNICATION INFORMATION:
TELEPHONE: 610-270-4478
TELEFAX: 610-270-5090
TELEX:
INFORMATION FOR SEQ ID NO: 508:
SEQUENCE CHARACTERISTICS:
LENGTH: 74 amino acids
TYPE: amino acid
; STRANDEDNESS: single
; TOPOLOGY: linear
; MOLECULE TYPE: No. 6348328e
US-08-858-207A-508

Black et al.

```
Query Match            20.3%;  Score 322;  DB 4;  Length 74;
Best Local Similarity  97.1%;  Pred. No. 1.2e-19;
Matches   66;  Conservative    1;  Mismatches    1;  Indels    0;  Gaps    0;

Qy        10 MKKKLLAGAITLLSVATLAACSKGSEGADLISMKGDVITEHQFYEQVKSNPSAQQVLLNM 69
Db         1 MKKKLLAGAITLLSVATLAACSKGSEGADLISMKGDVITEHQFYEQVKNNPSAQXVLLNM 60
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Qy        70 TIQKVFEK 77
Db        61 TIQKVFEK 68
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RESULT 9
US-09-134-001C-3811
; Sequence 3811, Application US/09134001C
; Patent No. 6380370
; GENERAL INFORMATION:
; APPLICANT: Lynn Doucette-Stamm et al
; TITLE OF INVENTION: NUCLEIC ACID AND AMINO ACID SEQUENCES RELATING TO STAPHYLOCOCC
; TITLE OF INVENTION: EPIDERMIDIS FOR DIAGNOSTICS AND THERAPEUTICS
; FILE REFERENCE: GTC-007
; CURRENT APPLICATION NUMBER: US/09/134,001C
; CURRENT FILING DATE: 1998-08-13
; PRIOR APPLICATION NUMBER: US 60/064,964
; PRIOR FILING DATE: 1997-11-08
; PRIOR APPLICATION NUMBER: US 60/055,779
; PRIOR FILING DATE: 1997-08-14
; NUMBER OF SEQ ID NOS: 5674
; SEQ ID NO 3811
; LENGTH: 330
; TYPE: PRT
; ORGANISM: Staphylococcus epidermidis
US-09-134-001C-3811

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Query Match            13.2%;  Score 210;  DB 4;  Length 330;
Best Local Similarity  27.5%;  Pred. No. 1.4e-09;
Matches   95;  Conservative   55;  Mismatches  112;  Indels   84;  Gaps   20;

Qy        23 SVATLAAC-SKGSEGAD--LISMK-GDV----ITEHQFYEQVKSNPSAQQVLLNMTIQKV 74
Db        19 SALLLGACGSNATESKDNTLISSKAGDVKVADVMKKMGKEQI-ANTS-----FSIVLNKV 72

Qy        75 FEKQYGSELDDKEVDDTIAEEKKQYG--ENYQRVLSQAGMTLETRKAQIRTS---KLVEL 129
Db        73 LADKYKDKVDTKDIDKDIKKEEKQYGGKDQFESMLKQQGMSLDDYKEQKKLSAYQKQLLL 132

Qy       130 AVKKVAEAELTDEAYKKAFDEYTPDVTAQI---IRLNNED-------KAKEVLEKAKAE- 178
Db       133 DKVNVSDKEIKENSKK----------TSHILIKVKSKSSDKEGLSDKKAKEKAEKIQKEV 182

Qy       179 ---GADFAQLAKDNSTDEKTKENGGEITFDSASTEVPEQVKKAAFALDVDGVSDVI-TAT 234
Db       183 EKNPNKFGEIAKKESMDSSSAKKDGSLGY-VIKGQMVDSFEKALFKLKEGEVSKVVKTDY 241

Qy       235 GTQAYSSQYYIVKLTKKTE---KSSNI-----------------DDYKEKLKTVILTQKQ 274
Db       242 G-------YHIIKADKETDFNSEKSNIKQKLIEEKVQKKPKLLTDAYKELLKEYKVDYKD 294

Qy       275 ND--STFVQSIIGKELQAANIKVKDQAFQNIFTQYIGGGDSSSSSS 318
Db       295 RDIKKAIEDSILDPD------KIKQQQQQ----QSQGGSGLTNSGS 330
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RESULT 10
US-09-252-991A-17422
; Sequence 17422, Application US/09252991A
; Patent No. 6551795
; GENERAL INFORMATION:
; APPLICANT: Marc J. Rubenfield et al.
; TITLE OF INVENTION: NUCLEIC ACID AND AMINO ACID SEQUENCES RELATING TO PSEUDOMONAS
; TITLE OF INVENTION: AERUGINOSA FOR DIAGNOSTICS AND THERAPEUTICS
; FILE REFERENCE: 107196.136
; CURRENT APPLICATION NUMBER: US/09/252,991A
; CURRENT FILING DATE: 1999-02-18
; PRIOR APPLICATION NUMBER: US 60/074,788
; PRIOR FILING DATE: 1998-02-18
; PRIOR APPLICATION NUMBER: US 60/094,190
; PRIOR FILING DATE: 1998-07-27
; NUMBER OF SEQ ID NOS: 33142
; SEQ ID NO 17422